

CHROMOSOME COMPLEX AT PREMEIOTIC ANAPHASE AND MEIOTIC METAPHASE

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With two text figures

A NUMBER of workers have reported in the case of plants that the individual chromosomes at anaphase are at least double. In this laboratory we have seen this duality in the root-tips (Dermen 1933), during all stages of microsporogenesis in *Rhoeo*, and in the telophase stage of embryo sac tetrad nuclei in *Lilium regale*. In the present paper we are presenting conclusive evidence indicating that this duality also exists in

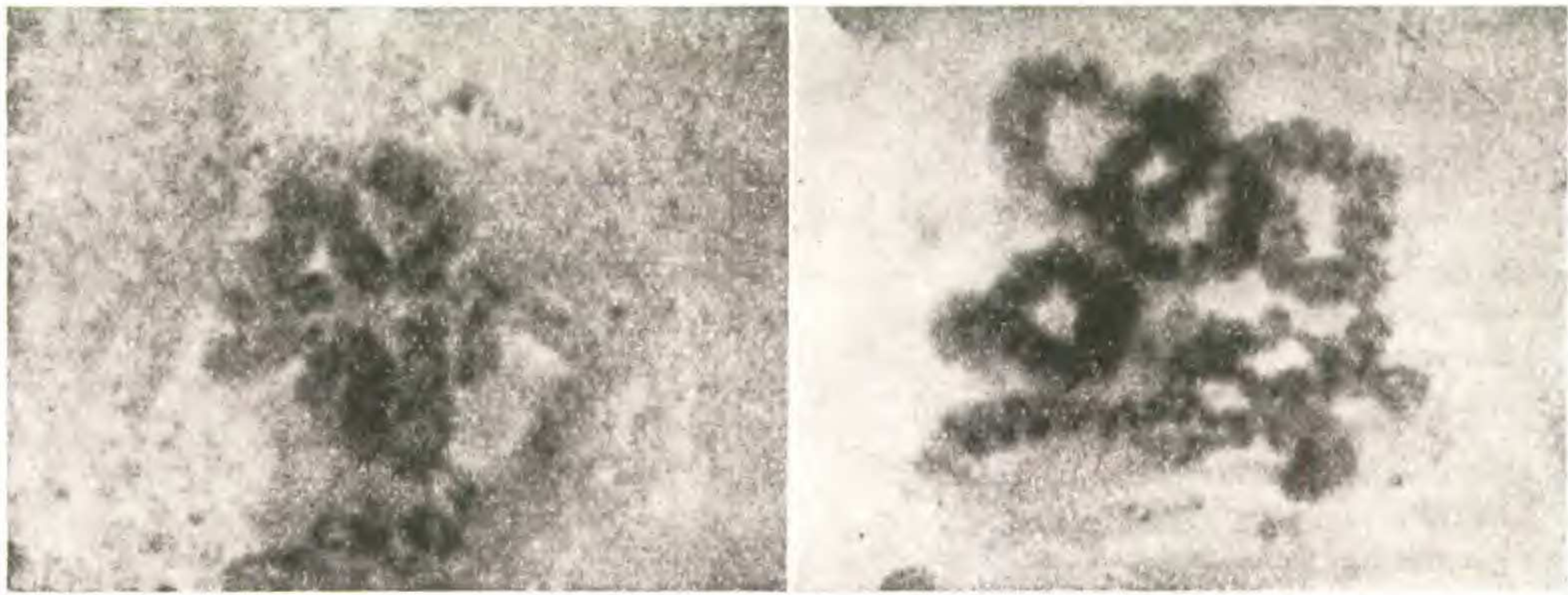


FIG. 1.

FIG. 2.

FIGURE 1. PREMEIOTIC ANAPHASE FROM *RHOEO DISCOLOR*.—FIGURE 2. MEIOTIC FIRST METAPHASE FROM A HYBRID *TRADESCANTIA* (*T. REFLEXA* X *T. PALUDOSA*).

the last premeiotic anaphase chromosomes which are destined to become meiotic leptotene threads. In addition to this the meiotic metaphase chromosomes were studied and were found to be structurally eight-parted.

The anthers of a young bud which is the third in series from that in the reduction stage in *Rhoeo discolor* were used for the study of premeiotic anaphase (Fig. 1). The cells of these anthers are for the most part in early prophase of meiosis but a few may be found in somatic metaphase or anaphase stage. The material showing the structure of meiotic first metaphase chromosomes (Fig. 2) was from a hybrid *Tradescantia*, *T. reflexa* X *T. paludosa*.

MATERIAL AND TECHNIQUE

The anthers in either case were smeared and pretreated for 5 to 10 seconds in 30% alcohol, 40 cc. in a staining jar, to which was added approximately 4 to 6 drops of ammonia water (26%), and the smear was then flooded with aceto-carmine 2 or 3 times for about 30 seconds. The slide was drained, any residual anther material that remained on the slide was removed, and a drop of aceto-carmine was added and a cover glass placed on the smear. The material was first located and studied, and if the preparation was successful the slide was gently heated over an alcohol lamp until small bubbles began to form. The cover glass was then pressed using a piece of filter paper for the purpose. To make these temporary preparations permanent either McClintock's acetic-alcohol or Metz's acetic-alcohol-xylol method may be used.

For a more permanent smear using the crystal violet-iodine stain, the following method is prescribed: Smear and pretreat as described above and place the slide, smear side down, in a small shallow dish containing a few drops of $\frac{1}{2}$ to $\frac{3}{4}$ strength Flemming's modified solution for $\frac{1}{2}$ to 1 minute. Rinse in water in a staining jar for one to two minutes, then stain in crystal violet for 15 seconds or longer depending upon the source of the material. Some plant smears may require over one minute of staining. If material so stained is thoroughly washed with xylol future destaining is prevented and the stain is as permanent as haematoxylin preparations.

DESCRIPTION

A photomicrograph of an anaphase genom at last premeiotic stage from *Rhoeo discolor* is shown in figure 1. It is one of the two anaphase groups seen from a slightly oblique polar view. It shows on the lower right the short arm of a chromosome pointing down. From this position the structure of this arm could be clearly made out indicating unmistakably that it is split. The halves seem to be independently coiled and are not intimately associated. Where these chromosomes are twisted they appear as if constricted.

A meiotic first metaphase from a hybrid *Tradescantia* (*T. reflexa* \times *T. paludosa*) is shown in figure 2, also a photomicrograph. A bivalent chromosome is seen in a horizontal plane. This pair of chromosomes at the connection point shows two kinds of split, one major and one minor. This type of opening was not confined to the rod-bivalents alone. In other cells in the ring-bivalent chromosomes at both ends of their connections, these double, large and small, diamond shaped openings could be observed, but for the purpose of illustration the rod-bivalent type was found best, for it is easily flattened out by pressure on the cover glass to show this feature.

DISCUSSION

The duality in the premeiotic chromosomes has been shown by Kaufmann (1926) in plant material. In animals, McClung (1928) and Robertson (1931) have demonstrated this duality of chromosomes at a corresponding stage. Koshy (1934) has illustrated the duality of premeiotic anaphase chromosomes and leptotene threads, though it appears from his drawings that he may have confused leptotene with pachytene stage.

Nebel (1932 and 1933), Stebbins (1935) and Goodspeed, Uber and Avery (1935) are in agreement that telophase chromosomes are four-parted. It is indicated that the leptotene threads, although optically single, are actually four-parted. Goodspeed et al. believe further, by estimation, that the meiotic metaphase chromosomes are sixteen-parted, thus homologizing with *Drosophila* salivary chromosomes which are suggested to be sixteen-parted.

Huskins (1932) and Huskins and Smith (1934 and 1935) have admitted the duality of anaphase chromosomes elsewhere but deny it in the premeiotic anaphase. They also have reported eight-parted meiotic metaphase chromosomes. Darlington (1935) on the other hand has denied the duality of anaphase chromosomes in all regions. He believes that anaphase chromosomes are single in both somatic and premeiotic phases because of the fact that leptotene threads appear to be single.

Our own evidence (Figs. 1 and 2) shows conclusively that premeiotic anaphase chromosomes are at least two-parted and eight-parted at meiotic metaphase. The indication is that mitotic and meiotic phases are essentially of the same fundamental nature, with this difference, that instead of each chromosome remaining as a unit and perpetually dividing as in mitosis, in meiosis similar chromosomes (homologues) pair together (barring the crossing-over phenomenon from the discussion) and during the first metaphase move to opposite poles. In mitosis homologues do not form intimate association while in meiosis their association is quite intimate and complex. The mitotic chromosomes are four-parted at metaphase, in meiosis they are eight-parted in the bivalents, and correspondingly there are four in each half of the bivalents.

I tried to analyze the same situation of duality in *Lilium* ovules where as in any other plant there is usually only one cell that becomes meiotic. The preparations I made (sectioned material) were of a somewhat advanced stage. However, there is one obvious thing that should be considered here, that this meiotic cell is a product of a somatic cell division. Here one cell only becomes meiotic while its sister cell continues the somatic cycle. It is hard to imagine if the anaphase chromo-

somes in the somatic cell are split, as Huskins admits (1932), how the anaphase chromosomes of one such group become normally split while those in the opposite group remain unsplit to justify Huskins' assumption that leptotene threads are single by origin. In both sister cells chromosomes are either split or unsplit since these halves are supposedly mirror images of each other. If the chromosomes were single in this stage there should be two cells alike and both meiotic. However, there is normally only one such cell. The obvious conclusion is that if chromosomes are split in the somatic cells at anaphase elsewhere, they must be split here too and that undoubtedly late premeiotic anaphase chromosomes of the egg-mother-cell are of a dual nature.

SUMMARY

Conclusive evidence is presented showing that last premeiotic anaphase chromosomes in microsporogenesis are split and that metaphase chromosomes in pollen-mother-cells are optically eight-parted. It is argued that the same is true in the ovule.

A method which enabled us to bring out the coiled structure in meiotic chromosomes is described in some detail. This method involves pretreatment of smear preparations with ammonia in alcohol.

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